

## New Odour Glands in *Xylocopa* Males (Hymenoptera: Apoidea: Anthophoridae)

B. O. SCHLUMPFERGER AND D. WITTMANN

Institut für Landwirtschaftliche Zoologie und Bienenkunde, Universität Bonn, Melbweg 42,  
53127 Bonn, Germany

---

**Abstract.**—Odour glands were found in all tergites in males of the neotropical *Xylocopa bimaculata* Friese and *X. nigrocincta* Smith as well as in the palaearctic *X. violacea* Linné. Furthermore odour glands were found in the fore, middle and hind legs of *X. bimaculata* and *X. nigrocincta*. Males of *X. bimaculata* and *X. nigrocincta* establish non-resource based territories to which they attract females by spreading secretions of the odour glands over their body. For this they brush with specialized hairs on their hind legs over the abdomen, which they extend in order to expose the pores of the odour glands. The function of glands in all three pairs of legs is discussed in comparison with similar findings of odour glands in other species of carpenter bees and leafcutter bees.

---

Territorial male carpenter bees that actively search for females establish two kinds of territories: at nesting sites or at flowering plants (Hurd and Linsley 1975, Alcock 1991). Such territories may cover an area of several square meters. Other males attract females with sex pheromones to territories which contain neither nests nor food plants, so called non-resource based territories. These territories may cover a space less than one cubic meter, in many cases they measure 10 to 15 cm<sup>3</sup>. In some species a single male establishes its territory alone, for example in a tree. In others conspecific males establish their non-resource based territories together in close vicinity, forming so called leks. In these cases the distance between single territories may be as short as one meter.

Males in non-resource based territories have to cope with two problems: they have to leave their territories in order to find nectar as energy for their long lasting territorial flights. In *Xylocopa nigrocincta* males dehydrate the nectar that is fed to them by the mother in the nest. Thereby they get rid of excess water, and with a

higher concentration of sugar they improve their energy budget. Of males with the same amount of sugar in their crop those fly longer in their territories which have diluted the sugar in small volumes of water (Wittmann and Scholz 1989).

Furthermore their success in attracting a female depends on the amount of pheromones secreted per time. This may be one of the reasons why males establish their territories close to each other in leks. The advantage of such leks is probably that together the males have better chances to attract a female.

Within the genus *Xylocopa*, odour glands have been described from the head (mandibular gland) and from the thorax (mesosomal gland) (Wheeler *et al.* 1976, Vinson *et al.* 1986). Furthermore odour glands were found recently in the fore legs of some Old and New World *Xylocopa* males (Wittmann and Blochtein 1995).

Males of *X. hisutissima* and *X. sulcatipes* have been reported marking their mating territories with the secretions of the mandibular glands (Velthuis and Camargo 1975 a & b, Hefetz 1983). Territorial males can identify conspecific males as intruders of

their territory by the secretions of the mandibular glands. In consequence this odour elicits defence behaviour in the owner of the territory (Velthuis and Camargo 1975 a & b). Mesosomal glands are male specific. The males frequently brush their legs over their body thereby spreading the odour (Vinson *et al.* 1986). The secretions of these glands are supposed to act as territorial pheromones in non-resource based territories (Gerling *et al.* 1989).

#### MATERIAL AND METHODS

All observations on behaviour were made between September and November 1995 in the natural habitat in the forest reservation area Pró-Mata of the PUC-University Porto Alegre (Brazil). The area is located in the northern highlands of Rio Grande do Sul, the Serra Geral (50°–51° W and 29°–30° S) in an elevation of ca. 900 m, about 150 km north of Porto Alegre.

For identification we used the key to subgenera by Hurd and Moure (1963). Furthermore the bees were compared with the collection of the Biological Research Station of the University of Tübingen/Germany at the PUC-University in Porto Alegre, Brazil. Critical species were identified by Dr. J. S. Moure.

To analyse the territorial behaviour males were filmed during the territorial flight with a Panasonic F15.

For SEM-analysis we used a Stereoscan 250 Mk2 and a Hitachi S-800. The bees, or parts of them, were macerated in 5 % KOH for 24 hours, after which they were dehydrated in 50–100 % ethanol and dried for 24 hours at 30°C. If glands with chitinized ducts are present, these structures remain after the mazeration.

To check the presence of volatile substances in the area of glandular pores we washed the abdomen of a freshly killed territorial male in pentane. The samples were analyzed with a Fisons MD-800 GC-MS on a fused silica column, DB-5 (15 m × 0.32 mm), the temperature was programmed from 80°C (for 2 min) to 200°C at 10°C/min.

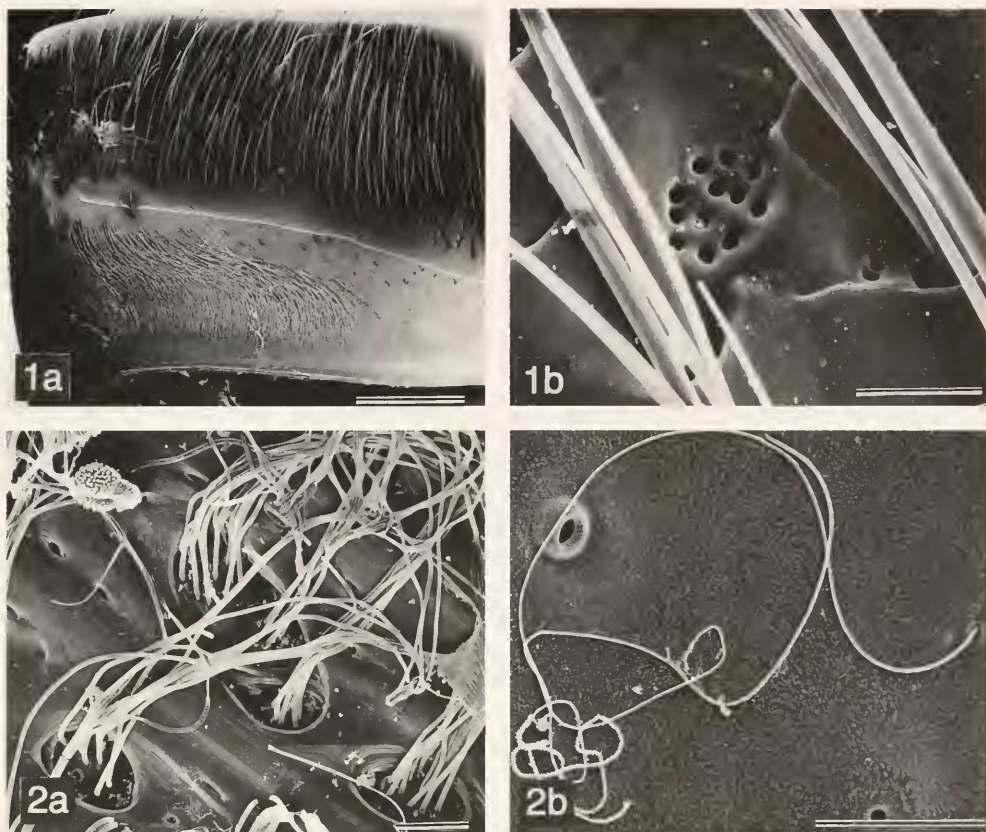
Compounds were identified by their mass spectra and using Kovats indices.

#### RESULTS

*Abdominal glands.*—SEM analyses of the sternites and tergites of *X. bimaculata* revealed that pores of odour glands are present in tergites I–VI. These pores are located on the frontal part of the tergites which are hidden under the anterior tergites (Fig. 1a). They are the openings of chitinous ducts which lead into glandular cells in the abdomen. After maceration these ducts are visible on the inner surface of the tergites. In tergites I–V we found the highest amount of pores. On tergite I and II the pores were scattered, while on tergites III, IV and V most of the pores appeared clumped. Between 10 and 20 pores were grouped in round areas with a diameter of 8–10 µm (Fig. 1b). The distances between these areas ranged between 20 and 40 µm. On the inner side of the tergite these groups of pores correspond with a bundle of cuticular ducts of odour glands (Fig. 2a). The terminal part of each duct is covered with short lateral ducts. This end apparatus is normally inside the glandular cell that has been macerated (Fig. 2b). In tergite VI only a few scattered pores were found, mostly on the lateral parts of the tergite.

*Description of hairs on the abdomen.*—Noteworthy are three different types of hairs on the tergites. On those parts of the tergites that are covered by the anterior tergites the hairs are plumose and about 200 µm long. The hairs on the posterior part of the tergite are unbranched and between 0.5 and 1 mm long. Most conspicuous are the bunches of bristles on the sides of the last three tergites.

*Abdominal glands in males of other Xylocopa species.*—We also found pores of odour glands in all tergites of *X. nigrocincta* and in *X. violacea*. In both species single pores have also a diameter of 2 µm. However, they are not arranged in groups as in *X. bimaculata*. The chitinous ducts of



Figs. 1–2. 1, Outer surface of *X. bimaculata* tergite IV: 1a, The median of the tergite is on the right borderline, the pores are hidden under the dense coverage of short hairs; 1b, Aggregation of 12 pores of odour glands. Each of the pores has a diameter of about 2 μm. 2, Inner side of the tergite IV: 2a, Bundles of cuticular ducts of odour glands, diameter of each duct is ca. 2 μm. 2b, Cuticular duct and the terminal apparatus. Scale bars: 1a) 1 mm, 1b) 10 μm, 2a) 20 μm, 2b) 40 μm.

the odour glands have the same appearance in all three species (Fig. 3a & b).

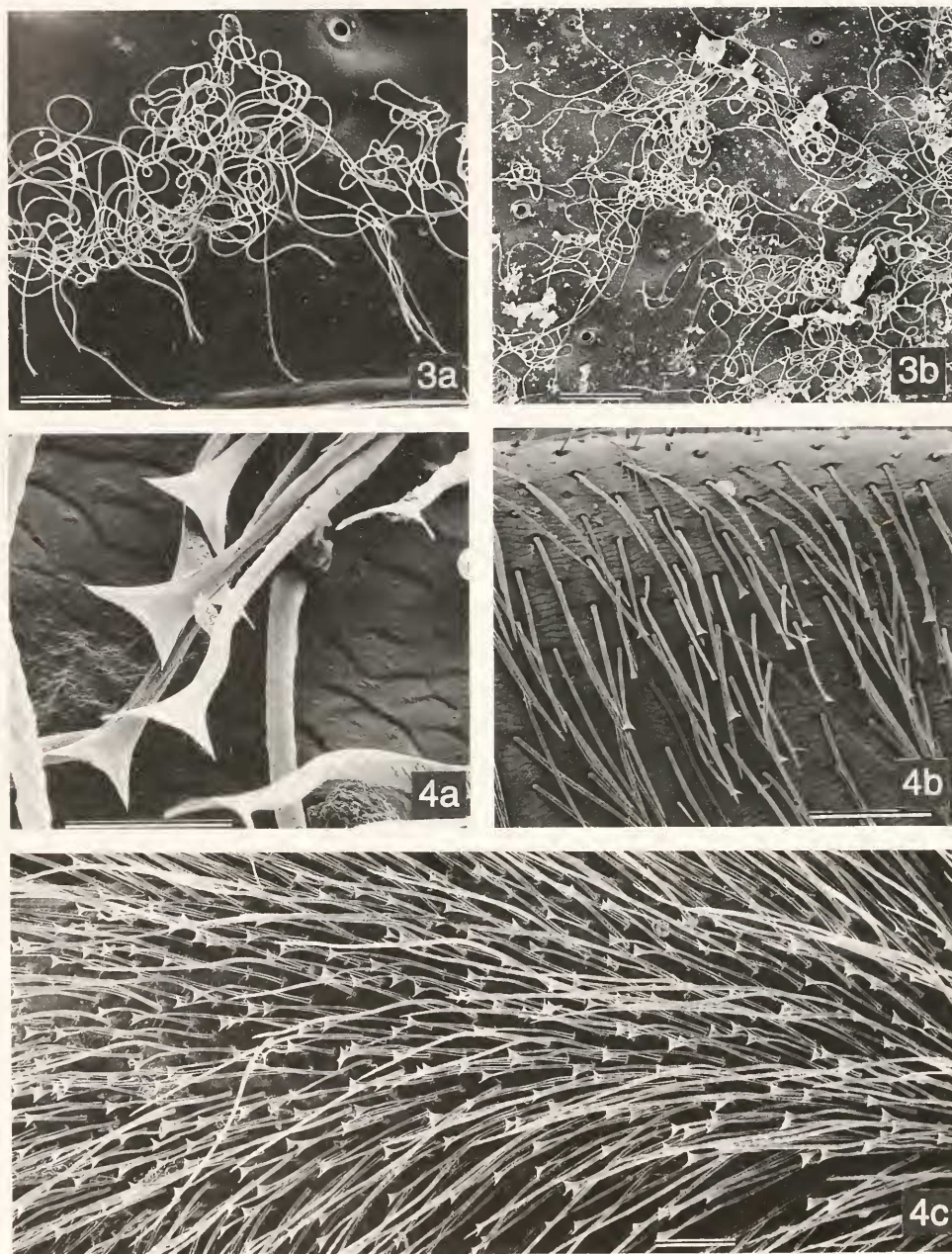
**Glands in legs.**—On the fore, middle and hind legs of *X. bimaculata* we found pores of odour glands. On the fore legs the pores were on the dorsal and anterior side of the basitarsus and also on the ventral side of the other tarsalia of the fore leg. On the middle leg pores were found on the dorsal side of the basitarsus, while on the hind leg the pores were found on the ventral side of the basitarsus. In *X. nigrocincta* pores were also found on the basitarsus of the fore leg.

**Hairs on the fore legs.**—A notable feature on the fore leg of *X. bimaculata* is a specific type of hair. When the male is viewed

frontally the hairs of the anterior side of the tarsalia and parts of the tibia appear shining white. The front leg bears on the anterior dorsal edge of the tarsalia a fan of bristles which are about three times as long as the diameter of the basitarsus. Such shiny white hairs are also present on the tarsi of the middle legs.

**Hairs on the hind legs.**—On the ventral side of femur and tibia of the hind legs we found a conspicuous type of hairs. They have smooth shafts and their tips are broadened and flattened forming a concave spatula (Fig. 4a). The concave side points to the surface of the cuticula. Besides these hairs the femur, tibia and the tarsi bear long pointed bristles with a





Figs. 3–4. 3, Chitinous odour ducts (with a diameter of ca. 2  $\mu\text{m}$ ). 3a, on the inner side of tergite I of *X. nigrocincta*; 3b, on the inner side of tergite II of *X. violacea*. 4, Hairs on the hind legs of male *X. bimaculata*. 4a, the widened and flattened tips form a concave spatula. 4b, hairs on the anterior side of the femur. 4c, hairs on the antero-ventral side of the tibia. Scale bars: 3a) 40  $\mu\text{m}$ , 3b) 100  $\mu\text{m}$ , 4a) 40  $\mu\text{m}$ , 4b) 200  $\mu\text{m}$ , 4c) 200  $\mu\text{m}$ .

rough surface. On the femur the spatula-like hairs are directed rectangular to the long axis of the femur (Fig. 4b) whereas on the tibia they are directed parallel to its

long axis (Fig. 4c). The function of this position of the hairs on the hind leg becomes clear when we look at the behaviour of *X. bimaculata* males in their territories.

**Territories.**—The mating period of *X. bimaculata* is from early October until late November. Due to the high altitude of the study site there is only one mating period. Males were recorded to fly in their territories between 06:15 and 16:30, the minimum temperature was 18°C. Individual territorial flights lasted up to 1.5 hours.

The territories of these males were always found at the very margins of the araucaria forest in non-flowering trees or shrubs. The males established their territories always on the sunny side of the forests and shifted them according to the position of the sun. They flew in territories with a radius of ca. 30 cm, sited between twigs or close to a bough, always in the shadow, positioned between 0.5 and 4 meters above the ground. The males kept their head downwind and changed their direction (not the position) according to the wind. Marked males were found to occupy the same territory on different days. However, this was not the rule.

In 1995 we observed 167 territories, 74% of them as single territories, in 26% 2–4 males established territories in close vicinity in the same tree. In 1994 we found leks with up to 10 males. The number of males in a lek changed frequently often within a few minutes. Sometimes the distance between the territories measured only 50 cm.

**Territorial behaviour.**—Within their territories the males of *X. bimaculata* hovered with loud buzzing mostly at one position, only shifting the direction from time to time to stay with the abdomen in the upwind position or in search for approaching females. Sometimes a male moved to establish the territory in another place without any recognizable reason. Males that left their territory probably to feed returned to their former or to a different position. In about 5% of the observed territorial flights males landed on the substrate within the territories. However, we could never observe them to rub their mandibles, their abdomen or legs over the substrate. Some of these males which had

landed were observed to brush their legs over their body.

While on wing in their territory the males held their fore and middle legs close to the body, so that the shiny white hairs on the fore and middle leg basitarsalia directed downwind and forward. The hind legs were stretched out backwards and away from the body. With high frequency (up to 11 times per minute) the males brushed with their hind legs from anterior to posterior over the dorsilateral parts of the abdomen, bending the abdomen downwards. They then rubbed the hind legs to each other and then to their middle legs. Less frequently they rubbed the middle legs to the fore legs and rarely the fore legs to the head. Towards the end of the territorial flight the males carried out these brushing movements with an ever decreasing frequency.

During the observation of 167 territories not a single female approached a male in its territory.

Males of *X. bimaculata* regurgitated and dehydrated nectar while hovering in the territory.

**Aggressions between males.**—Males in neighbouring territories have sometimes been observed to suddenly attack their neighbour. Some incoming males attacked territorial males immediately or were attacked by the hovering male. Sometimes the defending male left the territory to fly towards the intruder and hovered in front of him until one of them started to show antagonistic behaviour, including hits with the front legs, tumbling down while clinging to each other or chasing the opponent until both got out of sight of the observer.

Finally one male returned to the former territorial position, or in some cases even both males started to hover in close vicinity. Aggressive behaviour could be artificially initiated when we approached a dead male closer than 30 cm to a territorial male.

*Volatile substances in cuticular wash-*



ings.—In cuticular washings from the dorsilateral parts of the abdomen we could determine a series of alkanes with a chain length of 18 and longer as well as alkenes with the same chain length.

### DISCUSSION

Males of *X. bimaculata* were found to establish non-resource based territories, either alone or together with other males in leks. We could not observe that they mark substrate in their territories in order to attract females. Instead males were seen to brush their body with their legs. We suppose that while the hind legs brush over the abdomen they take up secretions of the odour glands and spread them during further movements over the plumose hairs over the abdomen and over the hind and middle legs. Good evidence for this is that the males bend their abdomen downwards and stretch it so that the pores of the odour glands are exposed. While the males move their hind legs over their abdomen femur and tibia are held in a 90° angle. The different exposition of the specialized hairs on femur and tibia ensures that they brush straight over the pores of glands. So the spatula-like tips can take up the secretions from the plumose hairs surrounding these pores and spread them subsequently over the long bristles on both sides of the abdomen and over the other legs. These movements and the high frequency with which they are carried out strongly suggest that the males perfume their body in order to attract conspecifics.

While the male is emitting these secretions he is facing downwind. This position possibly ensures that the male might see incoming females that follow an odour trace. The white areas in his face and the shiny white hairs on his middle and front legs could then serve as a further signal for approaching females to detect the male in its territory.

The chain length of the alkanes and alkenes we found in the cuticular washings suggests that they are not highly volatile.

Those substances may more likely serve as short range signals, maybe for mate acceptance in a female choice system or to detect the males at the margins of the forests. Further studies on the chemical properties of the gland secretions and their function during territorial and mating behaviour are necessary.

Unfortunately we could not observe copulations in *X. bimaculata*. Therefore, any further considerations on the function of the modified forelegs can only be hypothetical.

Anzenberger (1977) clearly observed that during copulation males of *X. (Mesotrichia) torrida* Westwood cover at least a part of the female's compound eyes with the fan of long bristles on their mid legs. Osten (1989) showed that in *Xylocopa* species from Africa and Sri Lanka such fans on fore legs also function as blind folds during copulation. These blind folds may have the effect that females stop flying when grabbed in mid air or prevent them to take off when mounted by a male on substrate.

The phenomenon that male Hymenoptera have blind folds and odour glands in modified forelegs was so far found in more than one hundred species of megachilid bees, in several species of neotropical and Old World carpenter bees and furthermore in a sphecid wasp (*Crabro cribrarius*) (Wittmann 1992, Wittmann and Blochtein 1995, Blochtein 1995).

Osten (1989) has described that during copula position in *X. perforator* males hold their basitarsi on the female's head. In these basitarsi Wittmann and Blochtein (1995) found odour glands. Combining both findings we strongly suggest that males during copula bring the secretions of the odour glands in close contact with the antennae of the female. Further students of *X. bimaculata* mating behaviour may therefore check whether such a copula position can also be found in these bees.

In contrary to male *X. nigrocincta*, which concentrate the nectar in the mother's nest

before each territorial flight (Wittmann and Scholz 1989), the males of *X. bimaculata* dehydrated nectar while hovering in the territory. We suppose that thereby male *X. bimaculata* improve their energy budget as has been shown for *X. nigrocincta*. While the latter are fed by their mother in the nest, the males of *X. bimaculata* collect the nectar by themselves and therefore have to evaporate the redundant water during the territorial flight.

### ACKNOWLEDGMENTS

This study was carried out in co-operation with the PUC-University, Porto Alegre, Rio Grande do Sul, Brazil, the University of Tübingen and the University of Bonn. We thank Dr. J.S. Moure for identification of the bees, Horst Schoppmann for skillful help at the scanning microscope and Andreas Jux for the GC-MS analysis.

### LITERATURE CITED

- Alcock, J. 1991. Mate-locating behaviour of *Xylocopa californica arizonensis* Cresson (Hymenoptera: Anthophoridae). *Journal of the Kansas Entomological Society* 64: 349–356.
- Anzenberger, G. 1977. Ethological study of African Carpenter bees of the genus *Xylocopa* (Hymenoptera: Anthophoridae). *Zeitschrift für Tierpsychologie* 44: 337–374.
- Blochtein, B. 1995. Die Bedeutung spezialisierter Beinstrukturen und Duftdrüsen der Männchen in der Paarungsbiologie von Blattschneiderbienen (Hymenoptera; Megachilidae). *Dissertation Universität Tübingen*, 96 p.
- Gerling, D., Velthuis, H. H. W. and Hefetz, A. 1989. Bionomics of the large Carpenter bees of the genus *Xylocopa*. *Annual Reviews of Entomology* 34: 163–190.
- Hefetz, A. 1983. Function of secretion of mandibular gland of male in territorial behavior of *Xylocopa sulcatipes* (Hymenoptera: Anthophoridae). *Journal of Chemical Ecology* 9: 923–931.
- Hurd, P. D. and Linsley, E. G. 1975. The principal *Larrea* bees of the southwestern United States (Hymenoptera: Apoidea). *Smithsonian Contributions to Zoology* 193: 1–47.
- Hurd, P. D. & Moure, J. S. 1963. A classification of the large carpenter bees (Xylocopini) (Hymenoptera, Apoidea). *University of California, Berkeley Publications in Entomology*, Vol. 29. 365 pp.
- Osten, T. 1989. Vergleichend-funktionsmorphologische Untersuchungen des Paarungsverhaltens von *Platynopoda* und *Mesotrichia* (Hymenoptera: Xylocopini). *Stuttgarter Beiträge zur Naturkunde, Ser. A*, 433, 18 pp.
- Velthuis, H. H. W. and Camargo, J. M. F. 1975a. Observations on male territories in a carpenter bee, *Xylocopa* (*Neoxylocopa*) *hirsutissima* Maidl (Hymenoptera, Anthophoridae). *Zeitschrift für Tierpsychologie* 38: 409–418.
- Velthuis, H. H. W. and Camargo, J. M. F. 1975b. Further observations on the function of male territories in the carpenter bee *Xylocopa* (*Neoxylocopa*) *hirsutissima* Maidl (Hymenoptera, Anthophoridae). *Netherlands Journal of Zoology* 25: 516–528.
- Vinson, S. B., Frankie, G. W. and Williams, H. J. 1986. Description of a new dorsal mesosomal gland in two *Xylocopa* species (Hymenoptera: Anthophoridae) from Costa Rica. *Journal of the Kansas Entomological Society* 59: 185–189.
- Wheeler, J. W., Evans, S. L., Blum, M. S., Velthuis, H. H. W., and Camargo, J. M. F. 1976. *cis*-2-Methyl-5-hydroxyhexanoic acid lactone in the mandibular gland secretion of a carpenter bee. *Tetrahedron Letters* 45: 4029–4032.
- Wittmann, D. 1992. Funktionsmorphologie der Vorderbeine von Megachiliden-Männchen. 13. Tagung der deutschsprachigen IUSSI-Sektion, Blaubeuren. 17 p.
- Wittmann, D. and Blochtein, B. 1995. Why males of leafcutter bees hold the females' antennae with their front legs during mating. *Apidologie* 26: 181–195.
- Wittmann, D. and Scholz, E. 1989. Nectar dehydration by male carpenter bees as preparation for mating flights. *Behavioral Ecology & Sociobiology* 25:387–391.